

CLAIM AMENDMENTS

In the claims:

Please amend claims 1 and 12, as shown below. Claims 22-25 are cancelled without prejudice. Claims 27 and 28 are new claims.

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1. (currently amended) A method for increasing the specific activity of a glycosyl hydrolase on a substrate, comprising replacing a hydrophobic surface binding amino acid of the hydrolase with a positively charged amino acid, to provide a mutant glycosyl hydrolase.

2. The method of claim 1, wherein the hydrophobic surface binding amino acid includes tryptophan or tyrosine and the positively charged amino acid is arginine.

3. A method for increasing the specific activity of a glycosyl hydrolase on a substrate, comprising replacing an active site associated glycosyl-stabilizing amino acid of the hydrolase with an amino acid, the replacing amino acid not strongly retarding cellobiose from leaving the active site to provide a mutant glycosyl hydrolase.

4. The method of claim 3, wherein the glycosyl-stabilizing amino acid comprises tyrosine 3 and the replacing amino acid comprises glycine.

5. The methods of claims 1 and 3 wherein replacing comprises site-directed-mutagenesis.

6. The methods of claims 1 and 3 wherein the mutant glycosyl hydrolase comprises a mutant EI endoglucanase.

7. The methods of claims 1 and 3 wherein the mutant glycosyl hydrolase comprises Y245G, Y42R, W82R, or a mixture thereof.

8. The methods of claims 1 and 3, wherein the substrate comprises pretreated biomass.

9. A mutant glycosyl hydrolase having enhanced catalytic activity, said mutant glycosyl hydrolase comprising an amino having a positively charged amino acid at a position occupied by a hydrophobic surface binding amino acid in a wild-type glycosyl hydrolase amino acid sequence, wherein said mutant glycosyl hydrolase has an enhanced catalytic activity of 10% to 50% compared to catalytic activity of the wild-type glycosyl hydrolase.

10. The mutant glycosyl hydrolase of claim 9 further defined as a cellulase. → *endo cellulase Cel5A*

11. The mutant glycosyl hydrolase of claim 9 further defined as Y245G.

A2 12. (currently amended) The mutant ~~glycosyl~~ glycosyl hydrolase of claim 9 further defined as Y42R.

13. The mutant glycosyl hydrolase of claim 9 further defined as a mannanase.

14. The mutant glycosyl hydrolase of claim 9 further defined as comprising W82R.

15 A method for converting a biomass into ethanol comprising: a. mixing a composition comprising biomass with a mutant glycosyl hydrolase having enhanced catalytic activity over a wild-type glycosyl hydrolase to provide a soluble fermentable sugar preparation; and b. fermenting said soluble fermentable sugar preparation to provide a composition comprising ethanol.

16. The method of claim 15 wherein the biomass is a cellulosic biomass.

17. The method of claim 15 wherein the mutant glycosyl hydrolase is Y245G.

18. The method of claim 15 wherein the mutant glycosyl hydrolase is Y82R.

19. The method of claim 15 wherein the mutant glycosyl hydrolase is W42R.

20. The method of claim 15 wherein the mutant glycosyl hydrolase comprises Y245G, Y82R, or W42R.

21. The method of claim 15 wherein the biomass is further admixed with a glycohydrolase.

22. (cancelled)

23. (cancelled)

24. (cancelled)

25. (cancelled)

26. A method for increasing the specific activity of a hydrolytic depolymerizing enzyme, comprising replacing an extended-active site residue that binds strongly to the leaving group with another that binds much less strongly to the leaving group.

A3 27. (new) A method for increasing the specific activity of a glycosyl hydrolase on a pretreated biomass substrate, comprising:

replacing, via site directed mutagenesis, a hydrophobic surface binding amino acid of the hydrolase with a positively charged amino acid arginine, the hydrophobic

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surface binding amino acid of the hydrolase selected from the group consisting of tryptophan and tyrosine, the hydrolase selected from the group consisting of an EI endoglucanase, Y245G, Y42R, W82R, and a mixture thereof; and

replacing, via site directed mutagenesis, an active site associated glycosyl-stabilizing amino acid of the hydrolase including tyrosine with an amino acid including glycine, the glycine not strongly retarding cellobiose from leaving the active site,

to produce a mutant glycosyl hydrolase having enhanced catalytic activity, the mutant glycosyl hydrolase comprising an amino having a positively charged amino acid at a position occupied by a hydrophobic surface binding amino acid in a wild-type glycosyl hydrolase amino acid sequence, wherein said mutant glycosyl hydrolase has an enhanced catalytic activity of 10% to 50% compared to catalytic activity of the wild-type glycosyl hydrolase.

28. The method of claim 27, further comprising steps of
mixing a composition comprising biomass with the mutant glycosyl hydrolase having enhanced catalytic activity over a wild-type glycosyl hydrolase to provide a soluble fermentable sugar preparation; and
fermenting the soluble fermentable sugar preparation to provide a composition comprising ethanol.
